FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. 100564-00103
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		DATE: February 25, 2002
		U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) 10/049633

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/08193	August 22, 2000	August 24, 1999 & March 31, 2000

TITLE OF INVENTION: IMMOBILISING AND LABELLING BIOPOLYMERS

APPLICANT(S) FOR DO/EO/US: Wilhelm ANSORGE (Gaiberg, Germany) and Konrad FAULSTICH (Wiesloch, Germany)

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
   (THE BASIC FILING FEE IS ATTACHED)

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper demand for International Preliminary Amendment was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English [35 U.S.C. 371(c)(2)].

7. ☒ Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].

9. ☐ An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].

Items 11 - 16 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: PCT/IPEA/409 in the German Language and PCT/ISA/210

U.S. APPLN NO. (IF KNOWN SEE 37 C.F.R. 1.5) NEW 10/049633	INTERNATIONAL APPLICATION NO. PCT/EP00/08193	ATTORNEY DOCKET NO. 100564-00103 DATE: February 25, 2002

17. ☒ The following fees are submitted:

Basic National Fee [37 C.F.R. 1.492(a)(1)-(5)]:

Search Report has been prepared by the EPO or JPO.....$890.00
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482).....$710.00
No international preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but international search fee paid to USPTO [37 C.F.R. 1.445(a)(2)].....$740.00
Neither international preliminary examination fee (37 C.F.R. 1.482) or international search fee [37 C.F.R. 1.445(a)(2)] paid to USPTO.....$1,040.00
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00

CALCULATIONS	PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(e)].				$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	46 - 20 =	26	X $ 18.00	$ 468.00	
Independent Claims	4 - 3 =	1	X $ 84.00	$ 84.00	
Multiple dependent claim(s) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1,442.00	
Reduction by one-half for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 C.F.R. 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 1,442.00	
Processing fee of $130.00 for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(f)]. +				$	
TOTAL NATIONAL FEE =				$ 1,442.00	
Fee for recording the enclosed assignment [37 C.F.R. 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 1,442.00	
				Amount to be refunded	$
				Charged	$

a. ☒ A check in the amount of $1,388.00 to cover the above fees is enclosed.
b. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive [37 C.F.R. 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
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Robert B. Murray
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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Wilhelm ANSORGE et al.

New U.S. National Stage of PCT/EP00/08193

Filed: Concurrently herewith Attorney Dkt. No.: 100564-00103

For: IMMOBILISING AND LABELLING BIOPOLYMERS

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

February 25, 2002

Sir:

Prior to calculation of the filing fees and initial examination of the application, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Before Line 1, page 1 insert

--CROSS-REFERENCE TO RELATED APPLICATION

This application is a National Stage entry of International Application No. PCT/EP00/08193, filed August 22, 2000, the entire specification claims and drawings of which are incorporated herewith by reference. --

IN THE CLAIMS:

Please amend claims 3-5, 8-12, 15, 16, 18-21, 24, 28-30, 35-37, 40 and 43 as follows:

3. (Amended) Method as claimed in claim 1,
characterized in that

the solid phase is selected from silicon, silicon dioxide, silicate glasses and silicon/silicon dioxide.

4. (Amended) Method as claimed in claim 1,

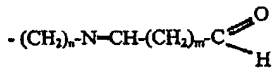
characterized in that

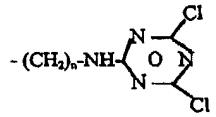
the solid phase comprises a structure of the general formula (I):

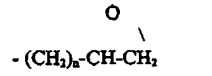
$$Z - R \qquad (I)$$

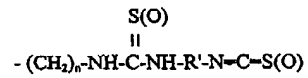
in which Z denotes silicon, silicon dioxide, a silicate glass or an oxidized silicon layer,

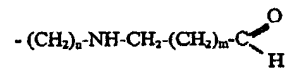
R denotes $(CH_2)_n$-C1



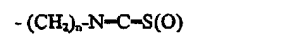








or



R^1 denotes an alkylene or arylene residue, in particular a 1,4 phenylene residue and

n and m each denote a positive integer preferably from 1 to 20.

5. (Amended) Method as claimed in claim 1,

characterized in that

the biopolymers are selected from nucleic acids and nucleic acid analogues.

8. (Amended) Method as claimed in claim 6;

characterized in that

X denotes $-(CH_2)_{n1}-$ or $(CH_2)_{n1}-O-\overset{\overset{O}{\|}}{P}-\overset{\overset{|}{OM}}{}$

in which

n^1 denotes a positive integer or 0, in particular from 1 to 20 e.g. 3, 6 or 12 and

M denotes hydrogen or a cation.

9. (Amended) Method as claimed in claim 6

characterized in that

the amino-modified nucleic acids are produced by enzymatic synthesis and

subsequent site-specific cleavage at the amino group.

10. (Amended) Method as claimed in claim 6,

characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (III):

$$Z-R^2-Y-X-NA \qquad (III)$$

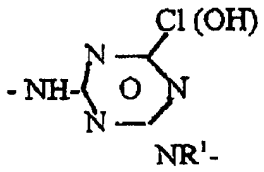
in which Z denotes a solid phase,

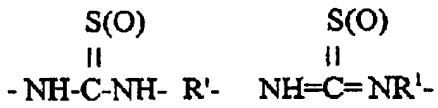
R^2 denotes - $(CH_2)_{n2}$-,

Y denotes - N=CH-$(CH_2)_m$-CH=N-,

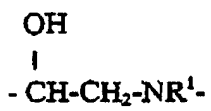
- $NH-CH_2-(CH_2)_M-CH_2-NR^1$,

- NR^1,





or



R', R^1, NA and X are defined as in claim 6,

n2 denotes a positive integer or 0, in particular from 1 to 20 e.g. 1, 3, 6 or 12

and

m denotes a positive integer preferably from 1 to 20.

11. (Amended) Method as claimed in claim 1,

characterized in that

biopolymers are applied to the solid phase in an array structure.

12. (Amended) Method as claimed in claim 1,

characterized in that

the biopolymers are applied by microinjection pipettes.

15. (Amended) Solid phase as claimed in claim 13,

characterized in that

the individual surface areas have a diameter of about 0.5 to 10 μm.

16. (Amended) Use of a solid phase produced as claimed in claim 1 to examine interactions between the immobilized biopolymers and free biopolymers.

18. (Amended) Use as claimed in claim 16,

characterized in that

the immobilized biopolymers are selected from nucleic acids, nucleic acid analogues and PNA and an interaction with free biopolymers based on hybridization is examined.

19. (Amended) Use as claimed in claim 16 for sequencing nucleic acids.

20. (Amended) Use as claimed in claim 16 for examining the expression of genes, the function of genes and metabolism.

21. (Amended) Device for carrying out examination of hybridization-based interactions of immobilized and free biopolymers comprising a solid phase produced as claimed in claim 1 hybridization probe, a hybridization buffer and a hybridization chamber optionally connected to a pumping device and a temperature control device.

24. (Amended) Use as claimed in claim 22,

characterized in that

hybridization probes are used which contain 5' amino-modified nucleotide building blocks.

28. Method as claimed in claim 26,

characterized in that

deoxyribonucleoside triphosphates labeled with fluorescent groups which are preferably selected from fluorescein, CY3 and CY5 are used in step (c).

29. Method as claimed in claim 26,

characterized in that

5' amino-modified nucleotide building blocks are incorporated into the cDNA molecules during the amplification.

30. (Amended) Method as claimed in claim 26,

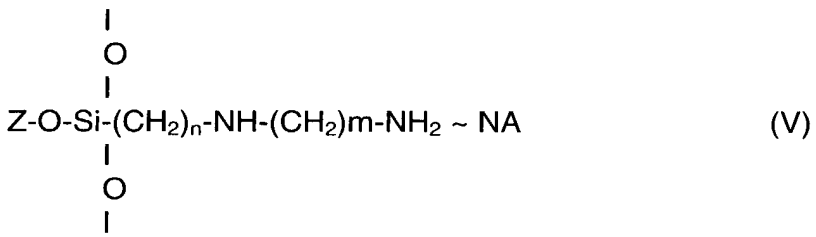
characterized in that

at least one of the primers used for the amplification in step (c) is a 5' amino-modified primer.

35. (Amended) Method as claimed in claim 31,

characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (V):



(V)

in which NA, Z, n and m represents a covalent or non-covalent interaction.

36. (Amended) Method as claimed in claim 31,

characterized in that

the biopolymers are applied to the solid phase in an array structure.

37. (Amended) Method as claimed in claim 31,

characterized in that

the biopolymers are applied by microinjection pipettes.

40. (Amended) Solid phase as claimed in claim 38

characterized in that

the individual surface areas a have diameter of about 0.5 to 10 μm.

43. (Amended) Method as claimed in claim 41 for mutation analysis.

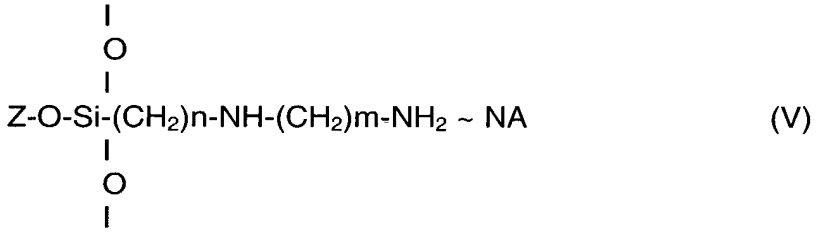
Please add the following new claims:

--44. Use of a solid phase produced as claimed in claim 13 to examine interactions between the immobilized biopolymers and free biopolymers.

45. Device for carrying out examinations of hybridization-based interaction of immobilized and free biopolymers comprising a solid phase produced as a solid phase as claimed in claim 14, at least one labeled hybridization probe, a hybridization buffer and a hybridization chamber optionally connected to a pumping device and a temperature control device.

46. Method as characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (V):



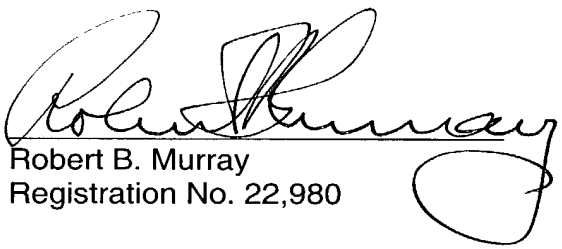
(V)

in which NA, Z, n and m are defined as claimed in claim 10 and ~ represents a covalent or non-covalent interaction.--

REMARKS

Claims 1-46 are pending in this application. By this Amendment, claims 3-5, 8-12, 15, 16, 18-21, 24, 28-30, 35-37, 40 and 43 are amended to correct the multiple dependency thereof and claims 44-48 have been added to place this application into better condition for examination. No new matter is added.

Respectfully submitted,



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MARKED-UP CLAIMS

3. (Amended) Method as claimed in claim 1 **[or 2]**,

characterized in that

the solid phase is selected from silicon, silicon dioxide, silicate glasses and silicon/silicon dioxide.

4. (Amended) Method as claimed in **[one of the previous]** claim**[s]** 1,

characterized in that

the solid phase comprises a structure of the general formula (I):

$$Z - R \qquad (I)$$

in which Z denotes silicon, silicon dioxide, a silicate glass or an oxidized silicon layer,

R denotes $(CH_2)_n$-C1

R^1 denotes an alkylene or arylene residue, in particular a 1,4 phenylene residue and

n and m each denote a positive integer preferably from 1 to 20.

5. (Amended) Method as claimed in **[one of the previous]** claim**[s]** 1,

characterized in that

the biopolymers are selected from nucleic acids and nucleic acid analogues.

8. (Amended) Method as claimed in claim 6 **[or 7]**;

characterized in that

X denotes $-(CH_2)_{n1}-$ or $(CH_2)_{n1}-O-\overset{O}{\overset{\|}{P}}-$

I
OM

in which

n^1 denotes a positive integer or 0, in particular from 1 to 20 e.g. 3, 6 or 12 and

M denotes hydrogen or a cation.

9. (Amended) Method as claimed in claim 6 **[to 8]**,

characterized in that

the amino-modified nucleic acids are produced by enzymatic synthesis and

subsequent site-specific cleavage at the amino group.

10. (Amended) Method as claimed in claim 6 **[to 9]**,

characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (III):

$$Z\text{-}R^2\text{-}Y\text{-}X\text{-}NA \qquad (III)$$

in which Z denotes a solid phase,

R^2 denotes - $(CH_2)_{n2}$-,

Y denotes - N=CH-$(CH_2)_m$-CH=N-,

- NH-CH_2-$(CH_2)_m$-CH_2-NR^1,

- NR^1,

R', R^1, NA and X are defined as in claim 6,

n2 denotes a positive integer or 0, in particular from 1 to 20 e.g. 1, 3, 6 or 12

and

m **[is defined as in claim 4]** denotes a positive integer preferably from 1 to 20.

11. (Amended) Method as claimed in claim 1 **[to 10]**,

characterized in that

biopolymers are applied to the solid phase in an array structure.

12. (Amended) Method as claimed in claim 1 **[to 11]**,

characterized in that

the biopolymers are applied by microinjection pipettes.

15. (Amended) Solid phase as claimed in claim 13 **[or 14]**,

characterized in that

the individual surface areas have a diameter of about 0.5 to 10 μm.

16. (Amended) Use of a solid phase produced as claimed in **[one of the]** claim**[s]** 1 **[to 12] [as claimed in one of the claims 13 to 15]** to examine interactions between the immobilized biopolymers and free biopolymers.

18. (Amended) Use as claimed in claim 16 **[or 17]**,

characterized in that

the immobilized biopolymers are selected from nucleic acids, nucleic acid analogues and PNA and an interaction with free biopolymers based on hybridization is examined.

19. (Amended) Use as claimed in claim**[s]** 16 **[to 18]**for sequencing nucleic acids.

20. (Amended) Use as claimed in **[one of the]** claim**[s]** 16 **[to 18]** for examining the expression of genes, the function of genes and metabolism.

21. (Amended) Device for carrying out examination of hybridization-based interactions of immobilized and free biopolymers comprising a solid phase produced as claimed in **[one of the]** claim**[s]** 1 **[to 12] [as claimed in one of the claims 14 to 16,]** hybridization probe, a hybridization buffer and a hybridization chamber optionally connected to a pumping device and a temperature control device.

24. (Amended) Use as claimed in claim 22 **[or 23]**,

characterized in that

hybridization probes are used which contain 5' amino-modified nucleotide building blocks.

28. Method as claimed in claim 26 **[or 27]**,

characterized in that

deoxyribonucleoside triphosphates labeled with fluorescent groups which are preferably selected from fluorescein, CY3 and CY5 are used in step (c).

29. Method as claimed in **[one of the]** claim**[s]** 26 **[to 28]**,

characterized in that

5' amino-modified nucleotide building blocks are incorporated into the cDNA molecules during the amplification.

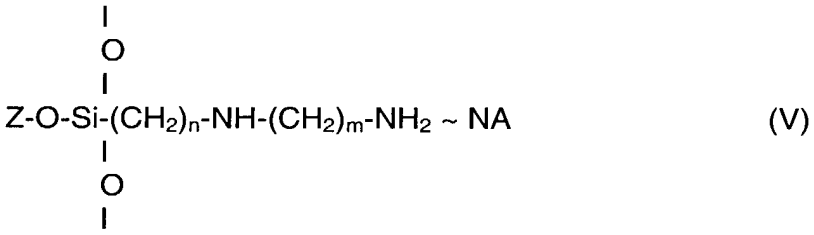
30. (Amended) Method as claimed in **[one of the]** claim**[s]** 26 **[to 29]**,

characterized in that

at least one of the primers used for the amplification in step (c) is a 5' amino-modified primer.

35. (Amended) Method as claimed in **[one of the]** claim**[s]** 31 **[to 34]**, characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (V):



$$Z\text{-}O\text{-}Si\text{-}(CH_2)_n\text{-}NH\text{-}(CH_2)_m\text{-}NH_2 \sim NA \qquad (V)$$

in which NA, Z, n and m represents a covalent or non-covalent interaction.

36. (Amended) Method as claimed in **[one of the]** claim**[s]** 31 **[to 35]**, characterized in that

the biopolymers are applied to the solid phase in an array structure.

37. (Amended) Method as claimed in **[one of the]** claim**[s]** 31 **[to 36]**, characterized in that

the biopolymers are applied by microinjection pipettes.

40. (Amended) Solid phase as claimed in claim 38 [**or 39**], characterized in that

the individual surface areas have a diameter of about 0.5 to 10 μm.

43. (Amended) Method as claimed in claim 41 **[or 42]** for mutation analysis.

MARKED-UP CLAIMS

3. (Amended) Method as claimed in claim 1 **[or 2]**,

characterized in that

the solid phase is selected from silicon, silicon dioxide, silicate glasses and silicon/silicon dioxide.

4. (Amended) Method as claimed in **[one of the previous]** claim**[s]** 1,

characterized in that

the solid phase comprises a structure of the general formula (I):

$$Z - R \qquad (I)$$

in which Z denotes silicon, silicon dioxide, a silicate glass or an oxidized silicon layer,

R denotes $(CH_2)_n$-C1

R^1 denotes an alkylene or arylene residue, in particular a 1,4 phenylene residue and

n and m each denote a positive integer preferably from 1 to 20.

5. (Amended) Method as claimed in **[one of the previous]** claim**[s]** 1,

characterized in that

the biopolymers are selected from nucleic acids and nucleic acid analogues.

8. (Amended) Method as claimed in claim 6 **[or 7]**;

characterized in that

X denotes $-(CH_2)_{n1}-$ or $(CH_2)_{n1}-O-\overset{O}{\overset{\|}{P}}-$

I
OM

in which

n^1 denotes a positive integer or 0, in particular from 1 to 20 e.g. 3, 6 or 12 and

M denotes hydrogen or a cation.

9. (Amended) Method as claimed in claim 6 **[to 8]**,

characterized in that

the amino-modified nucleic acids are produced by enzymatic synthesis and

subsequent site-specific cleavage at the amino group.

10. (Amended) Method as claimed in claim 6 **[to 9]**,

characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (III):

$$Z\text{-}R^2\text{-}Y\text{-}X\text{-}NA \qquad (III)$$

in which Z denotes a solid phase,

R^2 denotes - $(CH_2)_{n2}$-,

Y denotes - N=CH-$(CH_2)_m$-CH=N-,

- NH-CH_2-$(CH_2)_m$-CH_2-NR^1,

- NR^1,

R', R^1, NA and X are defined as in claim 6,

n2 denotes a positive integer or 0, in particular from 1 to 20 e.g. 1, 3, 6 or 12

and

m **[is defined as in claim 4]** denotes a positive integer preferably from 1 to 20.

11. (Amended) Method as claimed in claim 1 **[to 10]**,

characterized in that

biopolymers are applied to the solid phase in an array structure.

12. (Amended) Method as claimed in claim 1 [**to 11]**,

characterized in that

the biopolymers are applied by microinjection pipettes.

15. (Amended) Solid phase as claimed in claim 13 **[or 14]**,

characterized in that

the individual surface areas have a diameter of about 0.5 to 10 μm.

16. (Amended) Use of a solid phase produced as claimed in **[one of the]** claim**[s]** 1 **[to 12] [as claimed in one of the claims 13 to 15]** to examine interactions between the immobilized biopolymers and free biopolymers.

18. (Amended) Use as claimed in claim 16 **[or 17]**,

characterized in that

the immobilized biopolymers are selected from nucleic acids, nucleic acid analogues and PNA and an interaction with free biopolymers based on hybridization is examined.

19. (Amended) Use as claimed in claim**[s]** 16 **[to 18]**for sequencing nucleic acids.

20. (Amended) Use as claimed in **[one of the]** claim**[s]** 16 **[to 18]** for examining the expression of genes, the function of genes and metabolism.

21. (Amended) Device for carrying out examination of hybridization-based interactions of immobilized and free biopolymers comprising a solid phase produced as claimed in **[one of the]** claim**[s]** 1 **[to 12] [as claimed in one of the claims 14 to 16,]** hybridization probe, a hybridization buffer and a hybridization chamber optionally connected to a pumping device and a temperature control device.

24. (Amended) Use as claimed in claim 22 **[or 23]**,

characterized in that

hybridization probes are used which contain 5' amino-modified nucleotide building blocks.

28. Method as claimed in claim 26 **[or 27]**,

characterized in that

deoxyribonucleoside triphosphates labeled with fluorescent groups which are preferably selected from fluorescein, CY3 and CY5 are used in step (c).

29. Method as claimed in **[one of the]** claim**[s]** 26 **[to 28]**,

characterized in that

5' amino-modified nucleotide building blocks are incorporated into the cDNA molecules during the amplification.

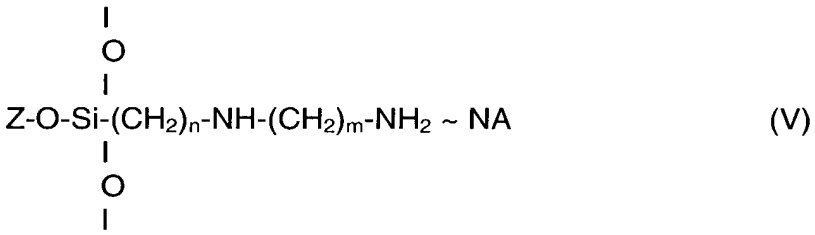
30. (Amended) Method as claimed in **[one of the]** claim**[s]** 26 **[to 29]**,

characterized in that

at least one of the primers used for the amplification in step (c) is a 5' amino-modified primer.

35. (Amended) Method as claimed in **[one of the]** claim**[s]** 31 **[to 34]**, characterized in that

after immobilization of the biopolymer the solid phase comprises a structure of the general formula (V):



$$Z\text{-}O\text{-}Si\text{-}(CH_2)_n\text{-}NH\text{-}(CH_2)_m\text{-}NH_2 \sim NA \qquad (V)$$

in which NA, Z, n and m represents a covalent or non-covalent interaction.

36. (Amended) Method as claimed in **[one of the]** claim**[s]** 31 **[to 35]**, characterized in that

the biopolymers are applied to the solid phase in an array structure.

37. (Amended) Method as claimed in **[one of the]** claim**[s]** 31 **[to 36]**, characterized in that

the biopolymers are applied by microinjection pipettes.

40. (Amended) Solid phase as claimed in claim 38 [**or 39**], characterized in that

the individual surface areas have a diameter of about 0.5 to 10 μm.

43. (Amended) Method as claimed in claim 41 **[or 42]** for mutation analysis.

Immobilising and labelling biopolymers

Description

The invention concerns methods for immobilizing biopolymers, in particular nucleic acids on a solid phase. In this process covalent bonds can be made between primary or/and secondary amine groups of the biopolymers and groups of the solid phase that react with amino groups. Alternatively the biopolymers can also be bound to the solid phase by non-covalent interactions.

The binding of nucleic acids to the surface of solid phases is a very critical step in the production of biochips. A current standard procedure includes the use of surfaces coated with polylysine on which DNA is immobilized by adsorption interactions the binding efficiency being increased by additional UV crosslinking. However, this method has considerable disadvantages with regard to the re-use of the chips, the binding capacity, the limitation to long DNA fragments, the damage to the DNA e.g. by cleavage of purine bases with formation of photodimers, the detachment of compounds and the signal background due to unspecific binding to the surface during the hybridization.

Hence there is a need to provide an effective and simple method for binding biopolymers, in particular DNA and oligonucleotides of any length, to solid phases in order to at least partially overcome the aforementioned disadvantages of the prior art.

According to the present invention a new method is provided for producing biochips and in particular nucleic acid chips which is based on the binding of biopolymers to a solid phase. In a preferred embodiment of this method, nucleic acids are immobilized which carry an amino group at their 5' end and are obtainable by enzymatic incorporation of 5'-amino-modified nucleoside building blocks into nucleic acids and

subsequent site specific cleavage of the nucleic acids. This method is described in detail in the International Patent Application PCT/EP99/02320 to which reference is expressly made in this connection.

A first aspect of the invention is a method for covalently immobilizing biopolymers on a solid phase comprising the steps:

(a) preparing a solid phase selected from metallic solid phases, oxidic solid phases and metallic-oxidic solid phases which contains groups on at least part of its surface which can react with amino groups and are selected from halogenide, aldehyde, epoxide, isocyanate and isothiocyanate groups,
(b) preparing a biopolymer with a reactive amino group and
(c) covalently immobilizing the biopolymer on the solid phase during which the reactive amino group of the biopolymer which is preferably a primary or/and secondary amino group forms a covalent bond with a reactive group of the solid phase.

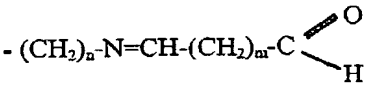
The groups of the solid phase that can react with amino groups are selected from halogenide groups, in particular alkyl or/and arylhalogenide groups, aldehyde groups, epoxide groups, isocyanate groups and isothiocyanate groups of which arylhalogenide, aldehyde and isocyanate groups are preferred. These reactive groups are produced by modifying the solid phase. The solid phase in turn is preferably selected from materials based on silicon such as silicon, silicon dioxide, silicate glasses or silicon/silicon dioxide.

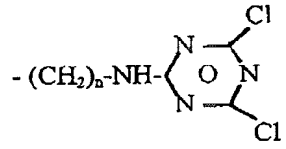
The activated solid phase prepared in step (a) of the method according to the invention preferably comprises a structure of the general formula (I):

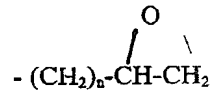
$$Z - R \qquad (I)$$

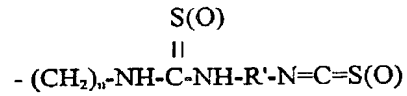
in which Z denotes silicon, silicon dioxide, a silicate glass or an oxidized silicon layer,

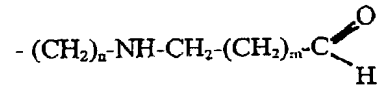
R denotes $(CH_2)_n$-Cl



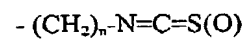








or



R' denotes an alkylene or arylene residue, in particular a 1,4 phenylene residue and n and m each denote a positive integer preferably from 1 to 20.

The biopolymers to be immobilized are preferably selected from nucleic acids, for example DNA molecules, RNA molecules or/and oligonucleotides, and nucleic acid analogues such as peptidic nucleic acids (PNA).

Amino-modified nucleic acids or nucleic acid analogues having a structure of the general formula (II) are particularly preferably immobilized on the solid phase:

$$R'NH\text{-}X\text{-}NA \qquad (II)$$

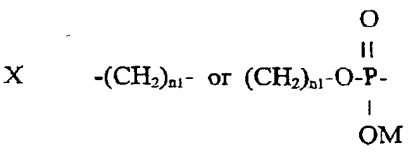
in which

R^1 denotes hydrogen or a C_1-C_6 alkyl group,

NA denotes a nucleic acid in particular a DNA or an oligonucleotide, or a nucleic acid analogue,

X denotes a chemical bond or a linker group and X is linked to the 5' or/and 3' terminal building block of NA.

NA particularly preferably denotes a nucleic acid and the group R^1NH-X is linked to NA via the 5' C atom of the 5' terminal sugar residue which is in particular a deoxyribose residue. X in turn preferably denotes



in which

n1 denotes a positive integer or 0, in particular from 1 to 20 e.g. 3, 6 or 12 and

M denotes hydrogen or a cation.

As described in PCT/EP99/02320 5' amino-modified nucleic acids can be produced by the enzymatic incorporation of 5' amino-modified nucleotide building blocks into nucleic acids and subsequent site specific cleavage at the amino group. The enzymatic incorporation can be carried out using enzymes selected from the group comprising DNA-dependent DNA polymerases, DNA-dependent RNA polymerases, RNA-dependent DNA polymerases, RNA-dependent RNA polymerases and terminal transferases. The T7 DNA polymerase or related enzymes such as T3 or SP6 DNA polymerase or modifications of these enzymes are particularly preferred.

The site specific cleavage at the amino group can take place by increasing the temperature e.g. to at least 37°C, adjusting acidic conditions e.g. pH $\leq$ 5, microwave treatment, laser treatment, e.g. with an infrared laser or/and by enzymatic digestion on the 3' side of the nucleotide containing the 5' amino group for example with

exonucleases or endonucleases or phosphodiesterases, e.g. 3'→5' snake venom phosphodiesterase.

It is also conceivable a linkage of X with the 3' terminal building block of NA.

The immobilisation of the amino functional biopolymers on the solid phase is preferably carried out under alkaline conditions e.g. at a pH value of 9 to 11. The biopolymers are contacted with the solid phase to be coated in a solution preferably at a concentration of 0.1 to 100 μM in particular 2 to 50 μM. An area to be coated preferably has a size of 0.1 to 100 mm^2 in many cases several areas on a solid phase being coated with the same or different biopolymers. After the coating e.g. by spotting, the solid phase is dried and subsequently incubated in an aqueous medium at an elevated temperature e.g. ≥ 40°C. Coated solid phases can be obtained in this manner that have a binding capacity of up to several 100 fmol of the biopolymer e.g. of an oligonucleotide per mm^2.

The solid phase can be a biochip which optionally contains several defined areas occupied with nucleic acids in the form of array arrangements. The solid phase preferably has a structure of the general formula (III):

$$Z\text{-}R^2\text{-}Y\text{-}X\text{-}NA \qquad \text{(III)}$$

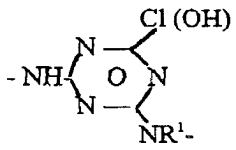
in which Z denotes the solid phase,

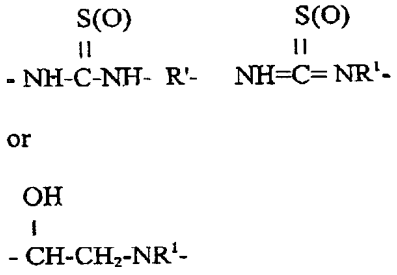
R^2 denotes $-(CH_2)_{n2}-$,

Y denotes $-N{=}CH\text{-}(CH_2)_m\text{-}CH{=}N-$,

$-NH\text{-}CH_2\text{-}(CH_2)_m\text{-}CH_2\text{-}NR^1$,

$-NR^1$,





or

R', R^1, NA and X are defined as above,

n2 denotes a positive integer or 0, in particular from 1 to 20 e.g. 1, 3, 6 or 12 and

m is defined as above.

Yet a further subject matter of the present invention is a solid phase with immobilized biopolymers comprising a structure of the general formula (III) and the use of the solid phase to examine interactions between immobilized biopolymers and free biopolymers which are preferably derived from biological samples and can for example be selected from nucleic acids, nucleic acid analogues, peptides, polypeptides, lipids and carbohydrates.

In a preferred embodiment of the present invention nucleic acids or nucleic acid analogues are used as the free biopolymers which contain 5' amino-modified nucleotide building blocks as described in PCT/EP99/02320. Due to the lability of the P-N-bond in these nucleic acids to temperature increases, acidic conditions, microwave treatment, laser treatment or/and enzymatic digestion, it is possible to selectively degrade the free biopolymers after the examination of interactions between the immobilized biopolymers and the modified free biopolymers is completed. This is of particular importance when using long nucleic acid fragments as immobilized biopolymers and free biopolymers in which the double-stranded hybridization regions can be larger than 100 bases and particularly preferably larger than 200 bases, e.g. 500 to 2000 bases. With such long hybridization regions it is usually only possible to detach the free biopolymers after completion of the examination under relatively drastic conditions which makes it difficult to re-use the

immobilized biopolymers for a rehybridization. These problems can be obviated by using nucleic acids with 5' amino-modified nucleotide building blocks as biopolymers. In this case a non-modified nucleic acid must be used as the immobilized biopolymer.

A second aspect of the invention is a method for covalently or non-covalently immobilizing biopolymers on a solid phase comprising the steps:

(a) preparing a solid phase selected from metallic solid phases, oxidic solid phases and metallic-oxidic solid phases which contains amino groups on at least part of its surface,

(b) preparing a biopolymer and

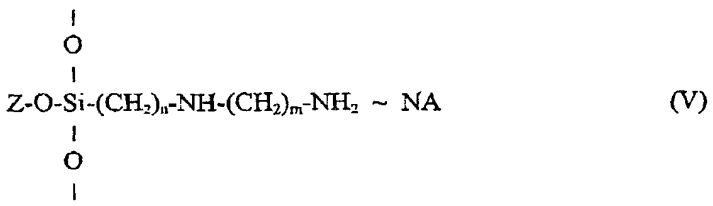
(c) immobilizing the biopolymer on the solid phase during which the solid phase containing amino groups forms stable covalent or non-covalent interactions with the biopolymer, in particular a nucleic acid, such as an unmodified nucleic acid or an amino-modified nucleic acid (as described above).

The amino groups on the solid phase are preferably produced by treating the solid phase surface with an aminosilyl compound. This aminosilyl compound preferably has a structure of the general formula IV:

$$(R^1O)_3Si\text{-}(CH_2)_n NH\text{-}(CH_2)_m NH_2 \qquad (IV)$$

in which R^1 denotes hydrogen or a C_1-C_3 alkyl group, preferably a methyl residue and n and m are defined as above. The compound of formula (IV) is particularly preferably N-(6-aminohexyl)-aminopropyl trimethoxysilane.

Binding of biopolymers results in a solid phase, for example a biochip, which optionally contains several defined areas occupied by nucleic acids in the form of array arrangements. The solid phase preferably has a structure of the general formula (V):



in which NA, Z, n and m are defined as above and ~ represents a covalent or non-covalent interaction.

The biopolymers to be immobilized are preferably applied to the solid phase by means of microinjection pipettes. These microinjection pipettes are for example glass capillaries which have an opening with a diameter of 0.1 µm to 1 mm, preferably 0.5 µm to 100 µm. By means of these microinjection pipettes extremely small surface areas containing immobilized biopolymers can be generated on the surface which leads to a considerable increase in the array density on the solid phase. The diameter of the individual surface areas on the solid phase is preferably 0.5 to 10 µm for example about 3 µm whereas only diameters of about 100 µm are usually achieved in the prior art. The improvement of the array density which is achieved by using microinjection pipettes is especially important for solid phases having structures of the general formulae (III) and (V). However, improvements are also achieved in array structures that have been produced in another manner.

Preferably interactions of the immobilized biopolymers with free biopolymers based on hybridization are examined. In order to achieve an optimal hybridization, a denaturation at an elevated temperature firstly takes place after contacting the coated solid phase with the free biopolymers and then incubation at the desired hybridization temperature for an adequate time period and using a suitable hybridization buffer. Preferred conditions for the hybridization especially of cDNA molecules to immobilized oligonucleotides are a hybridization temperature of 2 to 10°C, a hybridization duration of at least 4 h and a hybridization buffer which contains 1 to 50 mM divalent metal ions, in particular magnesium ions at a pH value of 7 to 9. The concentration of the free biopolymers e.g. cDNA molecules is

preferably 0.1 to 10 µm. After the hybridization it is washed at an adequate temperature for the desired stringency in each case (see Sambrook et al., Molecular Cloning, A Laboratory Manual (1989), Cold Spring Harbor Laboratory Press).

The solid phase can for example be used to sequence nucleic acids, to examine the expression of genes, the function of genes and metabolism. Other applications of the solid phase are the search for new active substances and drugs or effects thereof and optionally side effects thereof, the detection of genetically modified foods and the detection of mutations.

Yet a further subject matter of the invention is a device for carrying out examinations of interactions of immobilized and free biopolymers based on a hybridization comprising a solid phase according to the invention, at least one labelled hybridization probe, a suitable hybridization buffer and a hybridization chamber optionally connected to a pumping device and a temperature control device. The device can be used to detect the binding of labelled hybridization probes to immobilized biopolymers. Bound hybridization probes can be detached from the solid phase without loss of immobilized biopolymers which enables the device to be used for one or several additional hybridization cycles.

In a preferred embodiment a mixture of different cDNA molecules is used as the hybridization probe. This mixture of cDNA molecules can be obtained by a method which comprises the simultaneous amplification and labelling of cDNA molecules and comprises the steps:

(a) preparing RNA molecules, preferably a population of different RNA molecules e.g. total RNA, mRNA or other RNA fractions from a biological sample,
(b) reversely transcribing the RNA molecules using suitable primers without introducing marker groups into the resulting cDNA molecules,
(c) simultaneously labelling and amplifying the cDNA molecules using one or several labelled deoxyribonucleoside triphosphates and
(d) optionally purifying the resulting cDNA molecules.

The reverse transcription is preferably carried out by poly-dT priming using a poly-dT primer which additionally contains a section of a coding region under conditions such that additionally nucleosides are attached at the 3' end of the reversely transcribed cDNA (e.g. the Superscript II Reverse Transcriptase from GIBCO/Life Technologies, which can attach 3 C residues) and a corresponding complementary oligonucleotide (e.g. a SMART oligonucleotide containing a 5' GGG region from Clontech). It is preferably amplified by PCR using a primer complementary to the coding region and a nucleoside triphosphate mixture which contains one or several labelled triphosphates. The label can be a radioactive marker group. However, fluorescent groups are preferred such as fluorescein, CY5 and CY3.

The introduction of the marker groups during the amplification step has the advantage that large amounts of labelled probes are obtained. Other advantages of this procedure are high signal dynamics during the measurement and the possibility of using different marker groups.

In a particularly preferred embodiment 5' amino-modified nucleotides as described in PCT/EP99/02320 are additionally used during the amplification step as building blocks for the enzymatic synthesis of the amplification products. The amplification products produced in this manner contain labile P-N bonds which can be cleaved under defined conditions (see above). This facilitates the detachment of labelled hybridization probes from the solid phase after completion of the hybridization experiment which further increases the number of possible re-hybridization cycles for the solid phase.

Yet a further subject matter of the invention is a method for separating double-stranded nucleic acids due to their base sequence in which one of the nucleic acid strands forming the double-stranded nucleic acid fragments contains at least one 5' amino-modified nucleotide building block.

The separation of double-stranded nucleic acids due to their base sequence allows a distinction to be made among nucleic acid fragments of essentially the same length

which only differ in their base sequence. In such a separation the nucleic acid double strands are at least partially melted for example by a temperature gradient or a gradient of denaturing agents. The extent of this melting is determined under the given conditions by the strength of the base pairing between the two nucleic acids and hence depends on the specific nucleic acid sequence. Such methods are for example used for mutation analysis e.g. to analyse point mutations. When 5' amino-modified nucleotide building blocks are incorporated into one of the two nucleic acid strands, e.g. by using appropriate primers, the modified nucleic acid can be site-specifically cleaved each at a desired position by an appropriate increase in the temperature. The 5' amino-modified nucleotide building blocks are preferably incorporated at that position or at those positions where a mutation is assumed. Cleavage of the P-N bond at the 5' amino-modified nucleotide building block during separation by an appropriate matrix e.g. a gel medium or a liquid chromatography separation matrix under appropriately adjusted temperature conditions leads to a significant improvement of the separation behaviour and hence to an improved differentiation among different nucleic acid fragments of the same length but having different sequences.

The site-specific incorporation of 5' amino-modified nucleotide building blocks is preferably carried out by selecting combinations of suitable primers and 5' amino-modified nucleoside triphosphates and producing a modified complementary strand by primer extension.

The invention is further elucidated by the following examples.

Examples

1. Preparation of an activated solid phase using aromatic trisubstituted amines

1.1 Derivatization of glass surfaces

Standard microscope slides (Menzel) were purified for 1 h at room temperature with concentrated chromosulphuric acid and then washed with double distilled water.

Then the microscope slides were placed for 1 h at room temperature in 65 % nitric acid and then washed with double distilled water. Subsequently the microscope slides were placed for 1 h at room temperature in semi-concentrated hydrochloric acid and again washed in double distilled water.

After drying the microscope slides were treated for 3 h at room temperature with 1 % trimethoxy-3-amino-propylsilane in anhydrous toluene or dichloromethane or in methanol:water (1:1) and afterwards dried for 3 h at 110°C.

Derivatized microscope slides of the type CSA100 (CEL Associates, Texas, USA) can also be used for most applications.

1.2 Activation of the surfaces

2 g Cyanuric chloride was dissolved in 20 ml dry acetone and added to 180 ml dry N,N-dimethyl formamide (DMF). Subsequently 500 µl N,N-diisopropylethylamine was added. The microscope slides prepared according to 1.1 were placed for 30 min in this solution at room temperature and subsequently washed for 2 min with DMF and 2 min with acetone. After drying the microscope slides should be used immediately.

2. Preparation of an activated solid phase using dialdehydes

2.1 Derivatization of glass surfaces

Standard microscope slides (Menzel) were cleaned as described under 1.1 with chromosulphuric acid, nitric acid and hydrochloric acid and subsequently dried.

2.2 Activation of the surfaces

The microscope slides were placed in a 25 % solution of glutardialdehyde in double distilled water and allowed to stand in this solution for 24 h. Subsequently the microscope slides were removed from the solution and washed with double distilled water and dried.

3. Preparation of an activated solid phase using isocyanates

3.1 Derivatization of glass surfaces

Microscope slides (Menzel) were cleaned as described under 1.1 with chromosulphuric acid, nitric acid and hydrochloric acid and subsequently dried.

3.2 Activation of the surfaces

The microscope slides were contacted for 3 h at room temperature with 1 % 3-isocyanato-propyl dimethylchlorosilane in anhydrous toluene and subsequently dried.

4. Covalently bonding of DNA to activated glass surfaces

5'-(C_6)-amino-modified or 5'-(C_{12})-amino-modified oligonucleotides were manually spotted on the activated surfaces using a standard pipette. 0.1 M sodium carbonate pH 10 containing an oligonucleotide concentration between 2 and 50 µM was used for the spotting. Volumes of about 0.05 µl were applied to an area of about 1 mm^2.

The spotted oligonucleotides were dried and then placed in a hybridization chamber filled with distilled water and incubated there for about 1 to 4 h at 50°C. Subsequently the microscope slides were washed in water or buffer solution and dried.

5. Characterization of the binding of immobilized nucleic acids to the solid phase

The stability of the covalent binding of 5'-(C_6)-amino-modified or 5'-(C_{12})-amino-modified oligonucleotides to the solid phase was tested. Oligonucleotides were used for this which were labelled at the 3' end with fluorescein or CY5. The oligonucleotides bound to the activated surface were incubated in water or hybridization buffer at elevated temperatures for various time intervals.

The stability of the covalent immobilization was determined by means of the integral of the fluorescence signal. The amount of bound oligonucleotides was determined by

comparison with a calibration curve which was recorded by fluorescence scanning of known amounts of molecules.

The results for the stability of the covalent binding are shown in the following table:

Time	0	30	90	150	210
T=90°C	90.7	96.0	88.4	102.4	102.4
T=85°C	87.1	101.8	91.8	95.3	92.4
T=80°C	99.9	103.8	97.8	103.5	102.5

It is apparent that even after long incubations at an elevated temperature there is no significant detachment of the immobilized oligonucleotides.

The binding capacity of the solid phase is up to several 100 fmol oligonucleotide/mm^2.

6. Preparation of modified PCR products

A PCR was carried out using 5'-(C_6)-amino-modified or 5'-(C_{12})-amino-modified primers. These primers were prepared by chemical synthesis from commercially available synthesis building blocks by "standard amidite chemistry". Standard primers as well as gene-specific primers were used as primers. The PCR was carried out under standard conditions. The resulting modified PCR products were immobilized on a solid phase as described in section 4.

7. Simultaneous amplification and fluorescent labelling of cDNA pools with unknown sequences

Instead of introducing fluorescent labels during the reverse transcription, the RNA was firstly reversely transcribed into cDNA and subsequently simultaneously labelled and amplified in a second step.

The reverse transcription (RT) was carried out according to the SMART PCR cDNA synthesis kit (Clontech, Product information page 19).

A typical procedure is shown in the following.

Components of the reaction mixture:

- 4 µl cDNA (from RT)
- 33 µl water
- 5 µl 10 x cDNA PCR buffer (Clontech, SMART PCR cDNA synthesis kit)
- 6 µl nucleotide mix
- the PCR fluorescein labelling mix from Boehringer Mannheim is suitable for labelling with fluorescein;
- the following protocol was used for CY5 or CY3:

Compound	5 x conc. [nM]	Concentration of the stock solution [nM]	Added volumes [µl]
CY5-dUTP or CY3-dUTP	0.5	1	25
dTTP	1	100	0.5
dATP	2.5	100	1.25
dCTP	2.5	100	1.25
dGTP	2.5	100	1.25
Water	/	/	20.75
Σ			50

- 1 µl PCR primer (10 µM, Clontech, SMART PCR cDNA synthesis kit)
- 1 µl 50 x advantage cDNA polymerase mix (Clontech, SMART PCR cDNA synthesis kit)

The total reaction volume is 50 µl. The reaction was carried out in the following cycles: 95°C 1 min; 24 cycles: 95°C 15 s, 65°C 30 s and 68°C 8 min.

Example 8 Hybridization

The purified labelled probe (PCR product according to example 7 purified twice over Microcon membranes, preferably Microcon 100 membranes) was dissolved in 1 x annealing buffer (1 M Tris HCl pH 8.0; 100 mM $MgCl_2$ or 5 x SSC) at the desired concentrations (preferably as high as possible e.g. 25 mM). 10 to 25 µl of this solution was applied to the microscope slides prepared in example 4. Subsequently the microscope slides were sealed for example by covering them with another optionally hydrophobized (e.g. with trimethyl chlorosilane) microscope slide. After 1 h it was denatured at 80°C for at least 3 min and then incubated at the desired hybridization temperature for 4 to 36 h. Preferred conditions for the hybridization with oligonucleotides were 4°C for 8 to 12 h using solutions having concentrations of 2 µM oligonucleotide in 100 mM Tris HCl pH 8.0, 10 mM $MgCl_2$. Then it was washed in 0.1 x SSC at an adequate temperature for the desired stringency.

The fluorescence signals were detected using a Fuji FLA2000 fluoroscanner or a fluorescence microscope and a CCD camera.

When two different covalently bound primers (18-mers) with different sequences were used which are incubated with a solution of a fluorescent-labelled oligonucleotide which was completely complementary to one of the bound molecules and partially (30 %) complementary to the other molecule, a specific hybridization was only found with the completely complementary primer.

In order to test the reusability of the coated solid phases, the hybridized DNA molecules (oligonucleotides) were dehybridized by washing at 90°C for 20 – 60 min while gently shaking. The dehybridization was tested by fluorescence measurement. After complete dehybridization the chips no longer had a fluorescence signal and could be used for a second hybridization round. It was possible to reuse them several times (at least five times) without a significant loss of bound DNA molecules.

Example 9 Non-covalent immobilization

The binding of DNA to the solid phase does not have to be defined covalently but can also be carried out with N-(6-aminohexyl)aminopropyl trimethoxysilane. For this purpose the glasses to be modified were exposed to a 0.01-3 % solution of N-(6-aminohexyl)aminopropyl trimethoxysilane in methanol:water (= 1:1) and allowed to react for ca. 1-3 h at room temperature while gently shaking. Afterwards the glasses were washed thoroughly with methanol, water and again with methanol. Excess methanol was removed by centrifugation at 500 rpm and the glasses were allowed to react in an oven for ca. 3 h at 130°C. After cooling to room temperature the DNA to be applied (with or without 5'-amino modification) dissolved in water or buffer was added dropwise. The DNA was bound to the modified glass by a one hour exposure in a saturated water atmosphere at ca. 40 to 50°C and subsequent baking at 110°C for 10 to 15 minutes. After cooling to room temperature the DNA arrays manufactured in this manner are ready to be used for the remaining procedure (denaturation, hybridization, washing and detection).

Claims

1. Method for covalently immobilizing biopolymers on a solid phase comprising the steps:
   (a) preparing a solid phase selected from metallic solid phases, oxidic solid phases and metallic-oxidic solid phases which contains groups on at least part of its surface which can react with amino groups and are selected from halogenide, aldehyde, epoxide, isocyanate and isothiocyanate groups,
   (b) preparing a biopolymer with a reactive amino group and
   (c) covalently immobilizing the biopolymer on the solid phase.

2. Method as claimed in claim 1,
   characterized in that
   the groups on the solid phase that can react with amino groups are selected from arylhalogenide, aldehyde and isocyanate groups.

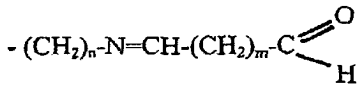
3. Method as claimed in claim 1 or 2,
   characterized in that
   the solid phase is selected from silicon, silicon dioxide, silicate glasses and silicon/silicon dioxide.

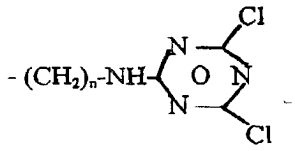
4. Method as claimed in one of the previous claims,
   characterized in that
   the solid phase comprises a structure of the general formula (I):

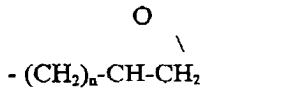
$$Z-R \qquad (I)$$

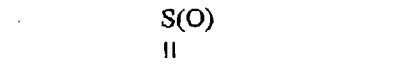
   in which Z denotes silicon, silicon dioxide, a silicate glass or an oxidized silicon layer,

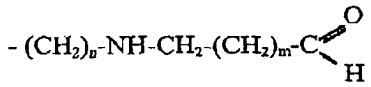
   R denotes $(CH_2)_n$-Cl



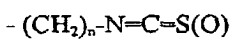








or



R' denotes an alkylene or arylene residue, in particular a 1,4 phenylene residue and

n and m each denote a positive integer preferably from 1 to 20.

5. Method as claimed in one of the previous claims,
characterized in that
the biopolymers are selected from nucleic acids and nucleic acid analogues.

6. Method as claimed in claim 5,
characterized in that
amino-modified nucleic acids or nucleic acid analogues having a structure of the general formula (II) are used

$$R^1NH\text{-}X\text{-}NA \qquad (II)$$

in which

R^1 denotes hydrogen or a C_1-C_6 alkyl group,

NA denotes a nucleic acid in particular a DNA or an oligonucleotide, or a nucleic acid analogue,

X denotes a chemical bond or a linker group and X is linked to the 5' or/and 3' terminal building block of NA.

7. Method as claimed in claim 6,

characterized in that

NA is a nucleic acid and the group R^1NH-X is linked to NA via the 5' C atom of the 5' terminal sugar residue which is in particular a deoxyribose residue.

8. Method as claimed in claim 6 or 7;

characterized in that

X denotes $-(CH_2)_{n1}-$ or $(CH_2)_{n1}-O-\overset{\overset{O}{\|}}{\underset{\underset{OM}{|}}{P}}-$

in which

n^1 denotes a positive integer or 0, in particular from 1 to 20 e.g. 3, 6 or 12 and

M denotes hydrogen or a cation.

9. Method as claimed in one of the claims 6 to 8

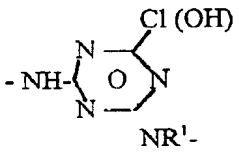
characterized in that

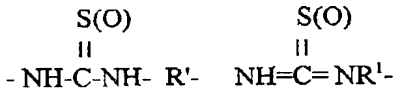
the amino-modified nucleic acids are produced by enzymatic synthesis and subsequent site-specific cleavage at the amino group.

10. Method as claimed in one of the claims 6 to 9,
characterized in that
after immobilization of the biopolymer the solid phase comprises a structure of the general formula (III):

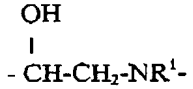
$$Z\text{-}R^2\text{-}Y\text{-}X\text{-}NA \qquad \text{(III)}$$

in which Z denotes a solid phase,
R^2 denotes $-(CH_2)_{n2}-$,
Y denotes $-N{=}CH\text{-}(CH_2)_m\text{-}CH{=}N-$,
$-NH\text{-}CH_2\text{-}(CH_2)_m\text{-}CH_2\text{-}NR^1$,
$-NR^1$,





or



R', R^1, NA and X are defined as in claim 6,
n2 denotes a positive integer or 0, in particular from 1 to 20 e.g. 1, 3, 6 or 12
and
m is defined as in claim 4.

11. Method as claimed in one of the claims 1 to 10,
characterized in that
biopolymers are applied to the solid phase in an array structure.

12. Method as claimed in one of the claims 1 to 11,
characterized in that
the biopolymers are applied by microinjection pipettes.

13. Solid phase with immobilized biopolymers comprising a structure of the general formula (III) as defined in claim 10.

14. Solid phase as claimed in claim 13,
characterized in that
it contains an array structure with several different biopolymers each on separate surface areas.

15. Solid phase as claimed in claim 13 or 14,
characterized in that
the individual surface areas have a diameter of about 0.5 to 10 µm.

16. Use of a solid phase produced as claimed in one of the claims 1 to 12 or a solid phase as claimed in one of the claims 13 to 15 to examine interactions between the immobilized biopolymers and free biopolymers.

17. Use as claimed in claim 16,
characterized in that
the free biopolymers are selected from nucleic acids, nucleic acid analogues, peptidic nucleic acids (PNA), peptides, polypeptides, lipids and carbohydrates.

18. Use as claimed in claim 16 or 17,
characterized in that
the immobilized biopolymers are selected from nucleic acids, nucleic acid analogues and PNA and an interaction with free biopolymers based on hybridization is examined.

19. Use as claimed in one of the claims 16 to 18 for sequencing nucleic acids.

20. Use as claimed in one of the claims 16 to 18 for examining the expression of genes, the function of genes and metabolism.

21. Device for carrying out examinations of hybridization-based interactions of immobilized and free biopolymers comprising a solid phase produced as claimed in one of the claims 1 to 12 or a solid phase as claimed in one of the claims 14 to 16, at least one labelled hybridization probe, a hybridization buffer and a hybridization chamber optionally connected to a pumping device and a temperature control device.

22. Use of the device as claimed in claim 21 in a method to detect the binding of hybridization probes to immobilized biopolymers.

23. Use as claimed in claim 22 comprising the detachment of bound hybridization probes from the solid phase and the use of the device for further hybridization cycles.

24. Use as claimed in claim 22 or 23,
characterized in that
hybridization probes are used which contain 5' amino-modified nucleotide building blocks.

25. Use as claimed in claim 24,
characterized in that
bound hybridization probes are subjected to a site-specific cleavage at the P-N bond of the 5' amino-modified nucleotide building blocks and are then detached from the biopolymers immobilized on the solid phase.

26. Method for the simultaneous amplification and labelling of cDNA molecules comprising the steps:
(a) preparing RNA molecules,
(b) reversely transcribing the RNA molecules without introducing marker groups into the resulting cDNA molecules,

(c) simultaneously labelling and amplifying the cDNA molecules using labelled deoxyribonucleoside triphosphates and

(d) optionally purifying the resulting labelled cDNA molecules.

27. Method as claimed in claim 26,
characterized in that
the RNA molecules prepared in step (a) contain a population of different RNA molecules, e.g. total RNA, mRNA or other RNA fractions from a biological sample.

28. Method as claimed in claim 26 or 27,
characterized in that
deoxyribonucleoside triphosphates labelled with fluorescent groups which are preferably selected from fluorescein, CY3 and CY5 are used in step (c).

29. Method as claimed in one of the claims 26 to 28,
characterized in that
5' amino-modified nucleotide building blocks are incorporated into the cDNA molecules during the amplification.

30. Method as claimed in one of the claims 26 to 29,
characterized in that
at least one of the primers used for the amplification in step (c) is a 5' amino-modified primer.

31. Method for immobilizing biopolymers on a solid phase comprising the steps:

(a) preparing a solid phase selected from metallic solid phases, oxidic solid phases and metallic-oxidic solid phases which contains amino groups on at least part of its surface,

(b) preparing a biopolymer and

(c) immobilizing the biopolymer on the solid phase during which the solid phase

containing amino groups forms stable covalent or non-covalent interactions with the biopolymer.

32. Method as claimed in claim 31,
characterized in that
the amino groups of the solid phase are produced by treating the solid phase surface with an aminosilyl compound.

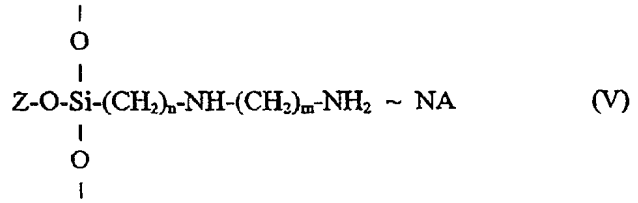
33. Method as claimed in claim 32,
characterized in that
the aminosilyl compound has a structure of the general formula IV:

$$(R^1O)_3Si\text{-}(CH_2)_n NH\text{-}(CH_2)_m NH_2 \qquad (IV)$$

in which R^1 denotes hydrogen or a C_1-C_3 alkyl group, preferably a methyl residue and n and m are defined as in claim 4.

34. Method as claimed in claim 33,
characterized in that
N-(6-aminohexyl)-aminopropyltrimethoxysilane is used as the compound of formula IV.

35. Method as claimed in one of the claims 31 to 34,
characterized in that
after immobilization of the biopolymer the solid phase comprises a structure of the general formula (V):



$$Z\text{-}O\text{-}Si\text{-}(CH_2)_n\text{-}NH\text{-}(CH_2)_m\text{-}NH_2 \sim NA \qquad (V)$$

in which NA, Z, n and m are defined as claimed in claim 10 and ~ represents a covalent or non-covalent interaction.

36. Method as claimed in one of the claims 31 to 35,
characterized in that
the biopolymers are applied to the solid phase in an array structure.

37. Method as claimed in one of the claims 31 to 36,
characterized in that
the biopolymers are applied by microinjection pipettes.

38. Solid phase with immobilized biopolymers comprising a structure of the general formula (V) as defined in claim 35.

39. Solid phase as claimed in claim 38,
characterized in that
it contains an array structure with several different biopolymers each on separate surface areas.

40. Solid phase as claimed in claim 38 or 39,
characterized in that
the individual surface areas have a diameter of about 0.5 to 10 µm.

41. Method for separating double-stranded nucleic acids due to their base sequence,
characterized in that
one of the nucleic acid strands forming the double-stranded nucleic acid fragments contains at least one 5' amino-modified nucleotide building block.

42. Method as claimed in claim 41,
characterized in that
the separation comprises a partial melting of the nucleic acid double-strands by a temperature gradient.

43. Method as claimed in claim 41 or 42 for mutation analysis.

Abstract

The invention concerns methods for immobilizing biopolymers, in particular nucleic acids on a solid phase. In this process covalent bonds are made between primary or/and secondary amine groups of the biopolymers and groups of the solid phase that react with amino groups.

Docket No. 100564-00103 ARENT FOX KINTNER PLOTKIN & KAHN, PLLC

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
(Insert Title) IMMOBILISING AND LABELLING BIOPOLYMERS

the specification of which is attached hereto unless the following box is checked:

☒ was filed on August 22, 2000 As PCT International Application
Number PCT/EP00/08193 and was amended on
and/or was filed on February 25, 2002 As U.S. Patent Application
Number 10/049,633 and was amended on

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

(List prior foreign applications)	(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
	199 40 077.6	Germany	24 August 1999	☒ Yes ☐ No
	100 16 073.5	Germany	31 March 2000	☒ Yes ☐ No
				☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)
(Application Number) (Filing Date)

☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

And I hereby appoint the firm of Arent Fox, Customer Number 004372 including as principal attorneys: Robert B. Murray, Reg. No. 22,980; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Douglas H. Goldhush, Reg. No. 33,125; Richard J. Berman, Reg. No. 39,107; Murat Ozgu, Reg. No. 44,275; Robert K. Carpenter, Reg. No. 34,794; Rustan Hill, Reg. No. 37,351; Kevin Turner, Reg. No. 43,437; Rhonda L. Barton, Reg. No. 47,271; Hans J. Crosby, Reg. No. 44,634, Brian A. Tollefson, Reg. No. 46,338; David D. Dzara, Reg. No. 47,543, Lynne D. Anderson, Reg. No. 46,412; Dinnatia J. Doster, Reg. No. 45,268, Michael A. Steinberg, Reg. No. 43,160 and Lynn A. Bristol, Reg. No. 48,898.

Please direct all communications to the following address:

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The undersigned hereby authorizes the U.S. attorneys named herein to accept and follow instructions from the undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be take in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the undersigned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

Full name of sole or first inventor Wilhelm ANSORGE

Inventor's signature _____ Date 21. Mai 02

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Full name of third inventor _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of forth inventor _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of fifth inventor _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of sixth inventor _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

#16

Docket No. 100564-00103

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
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(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
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The undersigned hereby authorizes the U.S. attorneys named herein to accept and follow instructions from the undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be take in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S attorneys named herein will be so notified by the undersigned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Wilhelm ANSORGE

Inventor's signature _____ Date _____

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Full name of second inventor Konrad FAULSTICH

Inventor's signature Konrad Faulstich Date June 6th, 2002

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Full name of third inventor _____

Inventor's signature _____ Date _____

Residence _____
Citizenship _____
Post Office Address _____

Full name of forth inventor _____

Inventor's signature _____ Date _____

Residence _____
Citizenship _____
Post Office Address _____

Full name of fifth inventor _____

Inventor's signature _____ Date _____

Residence _____
Citizenship _____
Post Office Address _____

Full name of sixth inventor _____

Inventor's signature _____ Date _____

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Citizenship _____
Post Office Address _____